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14. ABSTRACT
Assessment of axon health in spinal cord injury (SCI) is vital for proper diagnosis and treatment. Magnetic resonance imaging (MRI) is routinely performed in patients and provides valuable information about cord edema and hemorrhage. However, comprehensive prediction of axonal changes from in vivo MR imaging remains elusive. At the U. Penn site, we are applying two novel MRI methods to the problem of assessment of axonal loss, axonal diameter distribution, and myelin loss (q-space imaging (QSI) and ultra-short echo-time (UTE) MRI) first on animal specimens and then on human subjects. The objective during this period was to apply QSI to injured mouse spinal cords. We have already created the injured cords, and now with vital hardware upgrades complete, the QSI experiments will commence. Additionally, preliminary data on pig spinal cords was collected in a 1.5T scanner to assess clinical feasibility. The data are currently being analyzed. We have demonstrated for the first time the feasibility of UTE MRI to directly image myelin in freshly excised rat spinal cords. Through careful investigation of the MR signal of myelin extract, we validated our UTE MRI methodology. Our results suggest the potential of UTE MRI to quantify myelin content. Direct quantification of myelin content would remove ambiguities that exist in indirect methods leading to a more accurate assessment of myelin health.

15. SUBJECT TERMS
Axon Architecture, Spinal Cord Injury, Axon Loss, Myelin, Q-Space Imaging, UTE, MRI

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INTRODUCTION

Spinal cord injuries (SCI) produce direct mechanical disruption and the subsequent severe degeneration of axons, and are the processes underlying the associated neurologic deficits. Histological studies of fixed tissue in animal models of SCI have described axonal loss and demyelination occurring after trauma. Research at the U. Penn site brings novel magnetic resonance methodology to bear with the objective of obtaining quantitative information on axonal degeneration and myelin loss following spinal cord injury in a mouse model by pursuing the following specific aims per the work statement:

1. *We will perform q-space MR imaging (QSI) and simulations of QSI to quantify axonal architecture in healthy and injured mouse spinal cords.*
2. *We will quantify myelin content with three quantitative MRI techniques in healthy and injured mouse spinal cords and compare the results with histology.*

BODY

Specific Aim 1:

Normal and injured mice have been prepared for QSI analysis. A small pilot set of spinal cord specimens was prepared to refine the imaging approaches (normal & injured). Technical details have been worked out regarding injury placement, tissue collection and marking to enable reliable identification of lesion site and rostral/caudal orientation of the tissue specimens. We have generated healthy (n=4) and injured spinal cord tissue (2-day, 3-week and 3-month sacrifice, n=4 per time point).

There was an upgrade to the Bruker NMR/MRI system, which has delayed QSI experiments. Hardware modifications were needed to connect our previous custom gradient coil to the new system. The gradient coil also had to be optimized and recalibrated for the new system. As the old QSI pulse sequence program does not run on the new system, a new QSI pulse sequence program is currently under development.

Once the new pulse sequence program has been tested, QSI experiments will be performed to be followed by histologic analysis and QSI simulations.

Significant progress has been made toward translation of the QSI methodology to the clinic. Toward this goal a pulse sequence was designed and implemented for generating a series of images as a function of q (the spatial wave vector) at 1.5T on a clinical imager. Using this pulse sequence on fixed pig spinal cords, we have collected preliminary data to investigate the feasibility of using our previously published QSI methods on a clinical scanner.

Specific Aim 2:

Significant progress has been made towards 3D ultra-short echo-time (UTE) MRI of myelin. First, we succeeded in isolating bovine myelin and demonstrated that the spectroscopic and imaging characteristics of the hydrated myelin was identical to those obtained in situ in rat spinal cord. In the course of

these experiments the MR signal of myelin was studied extensively with proton, carbon and phosphorus NMR spectroscopy. The results of this pilot study indicate that UTE MRI may have potential for directly imaging myelin. We demonstrated the feasibility of a 3D dual-echo subtraction UTE sequence with adiabatic inversion long-T_2 suppression to directly image myelin in a freshly excised rat spinal cord (Figure 1). Lastly, we demonstrated a quantitative relationship between image-derived signal intensities and actual myelin concentration.

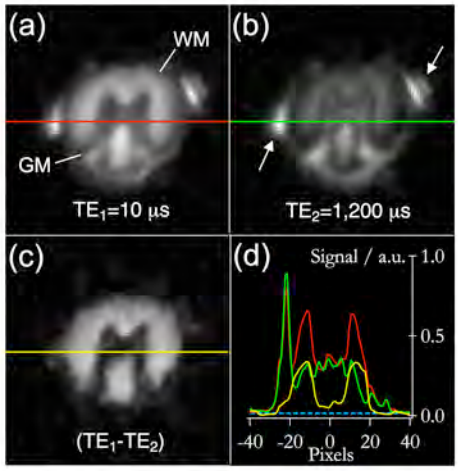

Figure 1. Sample 3D UTE images from rat thoracic spinal cords averaged over five central slices. Images obtained for a) TE=10 ms, b) TE=1,200 ms, and c) magnitude difference (maximum intensity range decreased by a factor of two to highlight myelin signal). D) Intensity profiles across the three images (delineated as red, green and yellow lines in panels a, b and c, respectively) to show relative white matter (WM), grey matter (GM) and background intensity. The dashed blue line represents the average noise level. WM and GM are indicated in panel a, and arrows highlight residual surface water in panel b.

Similar to Specific Aim 1, progress toward detecting myelin with IHMT and MR relaxometry has been hampered by the disruption in imaging capabilities caused by the upgrade of the Bruker Instruments micro-imaging system. However, as of the time of this report (10-15-11) software and hardware upgrades are complete and work should resume in November 2011.

KEY RESEARCH ACCOMPLISHMENTS

- Demonstrated feasibility of direct imaging of neural myelin as a new metric for the evaluation of spinal cord injury.
- Magnetic resonance characterization and feasibility demonstration of MR imaging of myelin *in situ* has been presented at the International Society for Magnetic Resonance in Medicine (ISMRM) Annual Meeting in Montreal, Canada (May 2011) and American Society for Neuroradiology Annual Meeting in Seattle (June 2011). See citations below.
- Submitted a manuscript on myelin MRI for publication to the Proceedings of the National Academy of Science.
- Generated the model injury mouse spinal cords.
- Tested the system upgrade of the Bruker Instruments micro-imaging system and interfaced custom-built gradients for high-resolution q-space imaging.

- Demonstrated the feasibility of translation of the q-space imaging technique in porcine model of the spinal cord on a 1.5T clinical imager and an abstract is being submitted for presentation at the ISMRM Annual Meeting in Melbourne, Australia in 2012.

REPORTABLE OUTCOMES

The new myelin imaging technique has shown potential for quantitative assessment of myelin content in the CNS of the rat spinal cord.

CONCLUSION

While the project is slightly delayed the progress made during the first year of the project gives the investigators confidence that the project will be completed in a timely fashion.

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2. Wilhelm MJ, Ong HH, Wehrli SL, Tsai P-H, Wright AC, Hackney DB, Wehrli FW. Prospects for quantitative imaging of myelin with ultra-short TE 3D radial MRI. 49th ASNR, Seattle, WA, June 4-9, (2011).

Prospects for quantitative imaging of myelin with dual-echo short inversion time 3D UTE MRI

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Introduction

Myelin is a lipid bilayer sheath encasing axons that enhances nerve conduction efficiency. Malformation or loss of myelin is at the core of many neurodegenerative disorders[1]. At present, there are few alternatives to destructive histologic methods to directly assess myelin. While MRI relaxometry and diffusion methods can indirectly assess myelin, they only detect myelin-associated water and not myelin itself. The short T_2^* of myelin protons and the presence of strong long T_2^* signals in white matter (WM) have thus far prevented direct myelin imaging with MRI. In this work, we examine the feasibility of ultra-short echo time (UTE) MRI to directly image myelin in purified myelin extracts and excised rat spinal cords (SC). NMR is first used to identify and characterize the MR signal from myelin. A dual-echo short inversion-time UTE sequence (de-STUTE) based on Ref. 2 is then used to suppress the long T_2^* signal and image a rat SC.

Methods

Rat and bovine SC samples were harvested from Sprague-Dawley rats (Charles River Labs) and a local butcher. A sucrose gradient method[3] was used to extract myelin from SC tissue using both WM and grey matter (GM). This method has been shown to preserve the bilayer structure of myelin. The myelin extract was suspended in D_2O (99.9% D, Sigma-Aldrich). Bovine myelin extract was used to prepare myelin/D_2O mixtures with varying myelin concentration (1.24-17.36 mg/ml). All spectroscopic and imaging experiments were performed on a 9.4 T vertical bore spectrometer/micro-imaging system (DMX-400, Bruker Instruments).

^{1}H NMR spectra were obtained for a freshly excised rat thoracic SC immersed in Fomblin (Sigma-Aldrich), as well as bovine and rat myelin extracts and samples with varying myelin concentration. Rat and bovine myelin extract was also dissolved in an organic solvent mixture to acquire high-resolution ^{1}H, and ^{31}P and ^{13}C proton-decoupled NMR spectra for identification of lipid components.

The rat thoracic SC section was imaged using a 2D de-STUTE sequence (128x128, FOV ~2cm). A 5ms adiabatic inversion pulse and 500ms inversion time were used to selectively invert and null long T_2^* signal. A 2D ramp-sampled UTE image with hard pulse excitation was then acquired with TE=10μs. Following a refocusing gradient, another 2D UTE image was acquired with TE=1200μs. A magnitude subtraction of the long TE image from the short TE image suppressed any residual long T_2^* signal.

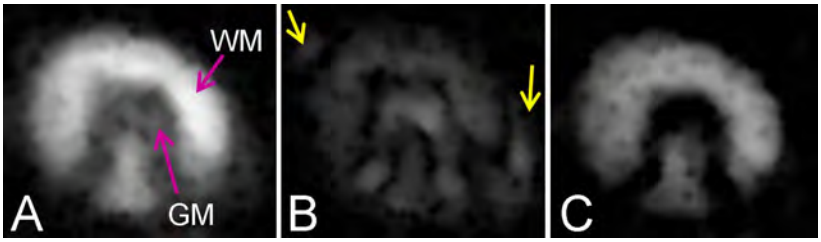

Fig 2. Linearly interpolated 2D de-STUTE images of excised rat thoracic SC section. A) TE=10μs. GM and WM are as indicated. B) TE=1200μs. Yellow arrows show residual surface water. C) Magnitude difference image of B from A.

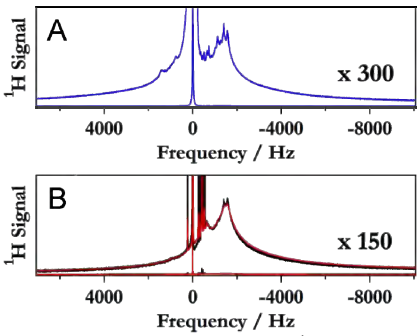

Fig 1. Full-scale and magnified ^{1}H spectra of intact rat SC (A) and myelin extract (B) for rat (red) and bovine (black). Spectra centered on water frequency. Note intact SC needed higher magnification to see broad peak.

Results and Discussion

Fig. 1 shows ^{1}H NMR spectra for intact rat SC and rat/bovine myelin extracts. The intact rat SC spectrum highlights the dominant water peak that would mask any myelin signal without long T_2^* suppression. The myelin extract spectra show residual sucrose and water peaks. The water peak is reduced as the myelin extract is mixed in D_2O. All three spectra share a non-Lorentzian, broad resonance (linewidth ~1700Hz) whose center is shifted ~3.5 ppm upfield from the water peak, which is consistent with the chemical shift of methylene protons in lipids. High-resolution ^{1}H, ^{31}P and ^{13}C spectra of myelin extract (not shown) are consistent with the galactolipid, phospholipid, and cholesterol constituents of myelin[4] and show negligible contributions from proteins. This strongly supports that the short T_2^* signal of SC consists predominantly of myelin lipids.

From its line width, T_2^* of myelin was estimated to be 100-200μs. Therefore, ramp-sampled UTE MRI should detect this signal with TE=10μs. Figure 2 shows de-STUTE images from a rat thoracic SC section. The short TE image shows good contrast between WM and GM. The long TE images shows a significant decrease in overall signal intensity implying that the short TE image contains a substantial amount of short T_2^* signal. The dark boundary between GM/WM and SC/water is the result of partial volume averaging of adjacent regions with different T_1s leading to destructive interference near the null time. The magnitude difference shows excellent suppression of GM and residual water outside the SC. The short T_2^* signal resides entirely in WM, which suggests that myelin is being imaged. Figure 3 shows ^{1}H spectra of various concentrations of myelin extract. The peak integrals are highly correlated with myelin extract concentration. The data highlight the potential of de-STUTE to quantify myelin concentration using a reference. Further work is needed to develop a 3D de-STUTE sequence and construct a reference phantom with similar relaxation properties to myelin analogous to the approach in Ref. 5.

Conclusion

This work examined the potential for de-STUTE to directly image and quantify myelin. NMR results indicates that the short T_2^* signal of SC is predominantly myelin lipids. de-STUTE images exhibit a short T_2^* signal present only in WM, which suggests the constituent imaged is indeed myelin.

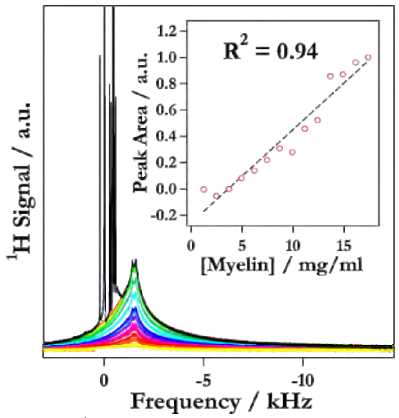

Fig 3. ^{1}H spectra for a series of bovine myelin extract concentrations. Residual sucrose and water peaks (black) were removed using a bi-exponential fit of the broad peak. Inset: Fitted broad peak area vs myelin concentration.

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